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1) Menzel et al. Chemiluminescence assay for choline acetyltransferase in tissue extracts by using immune absorption on monoclonal antibody. Anal. Chim. Acta. (1988) Vol. 205, Nos. 1-2, pp. 183-193.

2) Ebendal T. NGF in CNS: experimental data and clinical implications. Progress in Growth Factor Research. (1989) Vol. 1, No. 3, pp. 143-159.

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NGF IN CNS: EXPERIMENTAL DATA AND CLINICAL IMPLICATIONS

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The presence of β-nerve growth factor (NGF) and its cell surface receptor (NGF-R) in the brain has been well established by a variety of experimental techniques in recent years. In particular, the molecular cloning of NGF and NGF-R as well as the development of sensitive two-site ELISA techniques for determining the levels of NGF and antibodies to NGF-R suitable for immunohistochemistry have led to rapid accumulation of data in this field from many laboratories. A main finding is the function of NGF in the cholinergic neurons of the basal forebrain, expressing NGF receptors and responding to the factor by increased activity of choline acetyltransferase, and the production of NGF in cortical areas and hippocampus comprising terminal areas for the cholinergic projections from the basal forebrain. In addition, findings suggest that additional neurons in the brain and spinal cord may utilize NGF, notably during development and possibly also after lesion of the adult CNS. Moreover, observations indicate that endogenous levels of NGF are lowered in the aged rat brain concomitant with losses of NGF-dependent neurons in the basal forebrain. The involvement of NGF in human neurodegenerative diseases is not established but the application of NGF to degenerating cholinergic neurons in Alzheimer patients may prove useful. A promising approach to achieve this goal is the production of biologically active, recombinant NGF.

Keywords: Nerve growth factor, brain, NGF receptor, development, ageing, neurodegenerative disease.

INTRODUCTION

β-Nerve growth factor (NGF) is a basic protein of 118 amino acids with a well-established function as a target-derived neurotrophic molecule for sympathetic and some sensory peripheral neurons [1–3]. The pioneering studies on NGF by Rita Levi-Montalcini and Victor Hamburger in the early 1950s showed a marked stimulation on nerve fibre formation and neuron survival and maturation in the sympathetic and partly the sensory ganglia of the developing peripheral nervous system. In contrast no

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effects on CNS neurons were seen. The prominent developmental influence by NGF on peripheral noradrenergic neurons in the sympathetic part of the autonomic nervous system did also, however, in the early 1970s, lead to an interest of the effects of NGF on noradrenergic neurons within the brain. A detailed summary of the experiments and a discussion of the understanding in the field up to 1976 is found in a review paper by Freed [4], who cautiously concluded that "there is no evidence, at the present time, that NGF plays any role in CNS development". A function for NGF after brain lesion was, nevertheless, suggested [4] based on the findings by Björklund and collaborators [reviewed in 5] of enhanced growth of catecholamine fibres into intracerebral iris transplants after intracranial NGF injections. Later experiments by Levi-Montalcini and co-workers [6] in which NGF was injected locally near to the noradrenergic nucleus locus coeruleus in the brain stem did not, however, result in any response of these CNS neurons but evoked a massive ingrowth of sympathetic nerve fibres from the peripheral ganglia into the NGF-rich area of the brain stem.

Evidence against a function for NGF in central noradrenergic neurons was also obtained from experiments showing a lack of uptake and retrograde axonal transport in locus coeruleus neurons of radiolabelled NGF injected intracerebrally [7–9] as well as a lack of influence by NGF or anti-NGF antiserum on the fibre outgrowth from fetal locus coeruleus explanted for *in vitro* culture [10].

NGF AND FOREBRAIN CHOLINERGIC NEURONS

The first indications that NGF may affect a population of CNS neurons distinct from the catecholaminergic neurons were obtained from the injection experiments referred to above [8]. In addition to showing lack of uptake of NGF in locus coeruleus and substantia nigra neurons, the report demonstrated uptake and retrograde transport of NGF in septal neurons projecting to the hippocampus. A subsequent report [9] showed a similar uptake of NGF by neurons situated in the nucleus basalis from the neocortex of the rat, suggestive of cholinergic neurons in the basal forebrain utilizing NGF. In fact, an increase in choline acetyltransferase (ChAT) activity in fetal telencephalic cultures by the addition of NGF was reported in 1982 by Honegger and Lenoir [11]. Also, intraventricular injections of NGF in neonatal rats showed a substantial increase in ChAT activity in the septum, hippocampus and cortex [12]. In the adult rat, a smaller but significant increase in ChAT activity in the basal forebrain, were obtained after injections of NGF [12]. However, antibodies failed to reduce the ChAT activity [12], therefore leaving open the identity of a physiologically relevant trophic factor for these neurons.

A direct test addressing this was performed by Crutcher and Collins [13] who examined the presence of neurotrophic factors in the hippocampus using a bioassay for neurite extension in dissociated chicken neurons. Their results suggested the presence of at least two growth factors in the hippocampus, one of which was blocked by antibodies to NGF and thus was likely to represent this factor. This finding indicated that the hippocampus, at least in culture, can produce NGF.

The introduction of new techniques that will be reviewed here has recently confirmed the presence of NGF in the brain and has led to a drastic increase in our understanding of the distribution of NGF and NGF-receptor bearing neurons in the CNS. Several

papers provide reviews on different aspects of the rapid progress in this field [3, 14–17] and should be consulted for references not listed here.

DETERMINATION OF NGF mRNA IN CNS

The cloning of cDNA encoding the mouse NGF [18, 19] made it possible to determine by RNA blot hybridization the presence of NGF mRNA in polyA$^+$ RNA from different areas of the rat brain [20–22]. The first, independent reports, all showed the highest levels of NGF mRNA in the hippocampus followed by the cortex and olfactory lobe. Very low levels were found in septum and hypothalamus [21] whereas slightly higher levels were found in the cerebellum. Taken together, these observations indicated profound differences in the capacity to synthesize NGF between different regions of the brain. Only very low levels of NGF mRNA were found in the spinal cord of the rat [23].

These findings suggest strongly that NGF is not a general trophic factor in the brain but that its action is restricted to only some neuron populations in the CNS. The NGF distribution fits well with a major, but not necessarily exclusive, function for cholinergic neurons in the basal forebrain projecting to various cortical areas.

The mouse cDNA from the submandibular gland has also allowed for the isolation of genomic NGF clones from a number of species, including the human [19] and chicken NGF [24]. The human genomic NGF clone has made it possible to deduce the amino acid sequence of the human mature NGF protein (Fig. 1). Also, the distribution pattern with high levels of NGF mRNA in the cortical areas and hippocampus and low levels in the basal forebrain have been confirmed for the human brain [25]. In contrast, the phylogenetically divergent avian brain exhibited another distribution of NGF mRNA with very low levels of NGF mRNA in the cortical areas but high levels in the

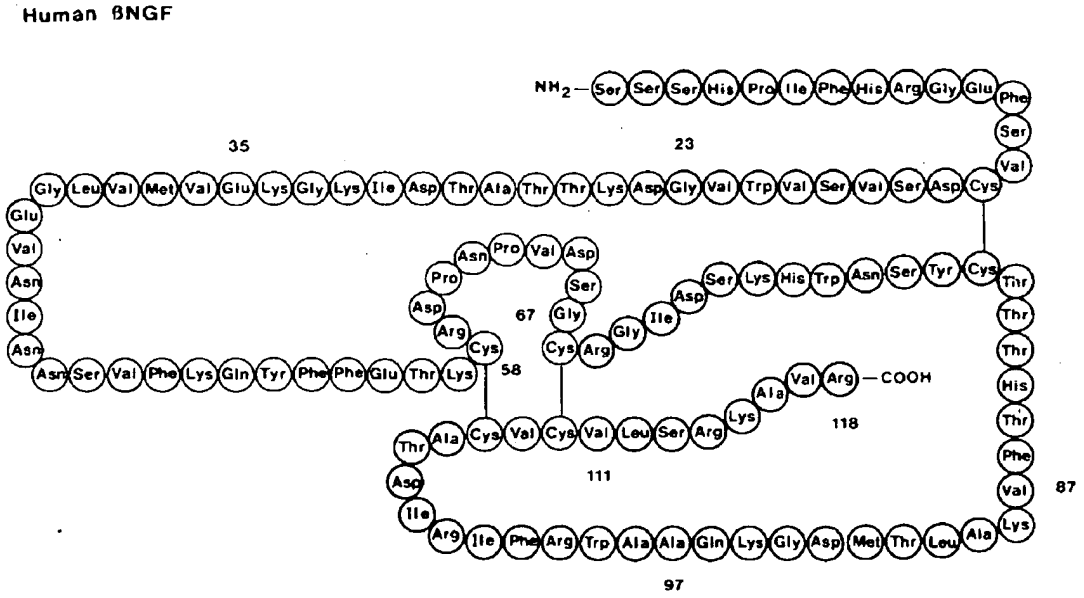

FIGURE 1. The amino acid sequence of the human NGF protein deduced from the cloned human NGF gene [19]. Disulphide bonds arranged in analogy with those of the mouse NGF (see Ref. 24).

optic tectum and brain stem [24]. These findings suggest similar functions for NGF in the brain of the rat and human but may indicate a difference in function for NGF in the avian brain.

The issue of which cells in the hippocampus are producing NGF has been addressed by *in situ* hybridization. Rennert and Heinrich [26] obtained labelling of the granular cell layer of the dentate gyrus and labelling of the pyramidal cell layer of the hippocampus, suggestive of production of NGF mRNA in neurons. This was confirmed in a study [27] with tritium-labelled probes allowing for autoradiographic resolution at the level of individual cells. This study established labelling of neurons in the pyramidal and granular neurons and confirmed the reduction in labelling of neurons after neurotoxic lesions by both *in situ* observations and RNA blots [27]. Thus, it appears highly likely that the major contribution to NGF in the hippocampus is by neurons receiving their cholinergic innervation from basal forebrain magnocellular neurons.

ANTIBODY DETECTION OF NGF IN BRAIN

A fruitful method in NGF research has been the introduction of sensitive two-site enzyme immunoassays (EIA) that allow for reliable determination of the low levels of NGF present in peripheral and CNS tissues [20, 21, 28]. These assays estimated the level of NGF in the rat hippocampus to 1–2 ng/g wet weight of tissue and in the cortex to 0.3–0.5 ng/g. Immunoaffinity chromatography of NGF from a large pool of rat brains resulted in yields of NGF active in a biological fibre outgrowth assay consistent with these levels of endogenous NGF [21]. This and the fact that several laboratories using different antibodies (monoclonal and polyclonal) reached similar figures for NGF concentration in brain areas speak strongly in favour for the authentic NGF being measured. Areas of the brain containing the somas of cholinergic neurons (septum, diagonal band of Broca, nucleus basalis of Meynert) contained around 0.4–0.7 ng/g indicating uptake and transport of NGF to these regions poor in NGF mRNA [21]. In contrast, the striatum, hypothalamus, hindbrain and optic tectum showed low levels of the NGF protein (0.1–0.2 ng/g tissue) [21] paralleling their low levels of NGF mRNA [21, 22]. It should be noted that the competitive RIAs used for NGF determinations prior to the introduction of the two-site EIAs falsely indicated high levels of NGF in many tissues.

Less consistent results have been obtained with the NGF antibodies applied for immunohistochemical localization of the factor. Ayer-LeLievre *et al.* [29] reported the presence of weakly immunoreactive nerve bundles in the adult rat iris and Whittemore *et al.* [21] found staining of many major fibre pathways in the adult rat brain as well as a fine network of immunoreactive nerve fibres in the cortex using the same affinity-purified antibodies as for the two-site EIA. Even though many appropriate controls for the purity and specificity were carried out on these antibodies, binding to epitopes on proteins other than NGF cannot be excluded. It is worth noting that different antisera, each with high titres for NGF in EIAs and excellent for immunohistochemical localization of NGF in the mouse submandibular gland [29], give individual staining patterns in the rat brain and indicate levels of NGF well above those expected from the EIA results [21, 28]. Thus caution should at present be applied in the interpretation of such immunohistochemical observations of NGF in the brain. A promising approach

to improve the reliability of the method may be the use of antibodies to different, non-overlapping synthetic peptides predicted from the sequence of the mature NGF and its precursor. In any case, as described above, the specificity of *in situ* hybridization made it possible to firmly establish the NGF synthesizing cells in the hippocampus [27] by their content of hybridizing NGF mRNA rather than by immunohistochemical visualization of the NGF protein itself.

NGF BINDING AND NGF RECEPTORS IN THE ADULT CNS

NGF binding to sections of the adult rat brain was first reported by Richardson *et al.* [30]. Their observations established selective, high-affinity binding of radiolabelled NGF to perikarya probably of cholinergic neurons in the basal forebrain (medial septal nucleus, diagonal band of Broca, lateral preoptic area and ventrocaudal globus pallidus) and, in addition, to neurons in the caudate-putamen, the medulla oblongata, the ventral cochlear nucleus and the dorsal nucleus of the lateral lemniscus. In the spinal cord, no labelled perikarya were seen [30]. The binding kinetics suggest the expression of NGF-R on the basal forebrain cholinergic neurons.

A number of studies have since established the actual presence of the NGF-R protein on these cells with the use of monoclonal antibodies to the rat and human NGF receptors. Hefti *et al.* [31] reported that the human brain contained NGF-R immunoreactive neurons in the medial septal nucleus, the diagonal band of Broca and the nucleus basalis of Meynert and, furthermore, demonstrated co-staining of these cells for acetylcholinesterase. Similar results were obtained in studies on the NGF-R immunoreactivity in the adult rat brain [32–34].

A particular problem has been to verify the NGF binding in the caudate-putamen [30] with immunohistochemical data for the NGF-R distribution. It may be that not all cholinergic interstriatal neurons express the NGF-R and that the NGF-R is expressed only at low levels in these cells in the adult brain. Recent immunohistochemical

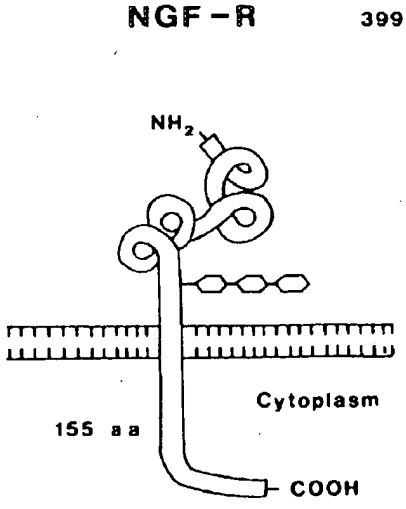

FIGURE 2. A schematic illustration of the NGF receptor based on sequence information from cloned cDNAs coding for the rat and human NGF-R [36, 37].

descriptions of a low number of NGF-R in the primate [35] and rat [34] putamen lend support to both possibilities.

The molecular cloning of the human [36] and rat [37] NGF receptor (Fig. 2) has also made possible the examination of NGF-R mRNA expression in the brain. RNA blot hybridization showed high levels of NGF-R mRNA in the rat septum and striatum but also revealed clearly detectable levels in the cerebellum and medulla oblongata [38]. *In situ* hybridization with an NGF-R probe also demonstrated distinct hybridization with magnocellular neurons in the rat basal forebrain [27] in accordance with binding data for NGF [30] and NGF-R immunohistochemistry [32–34].

EFFECTS OF NGF ON CNS NEURONS

An important finding was the increased activity of choline acetyltransferase (ChAT) in the basal forebrain of neonatal rats seen after injection of NGF [12]. Thus, a vital action of NGF is to increase the synthesizing enzyme for acetylcholine in the basal forebrain neurons. Similar selective increases in ChAT activity were seen also in the striatum of neonatal rats given NGF injections [39]. Moreover, an increased ChAT activity was, as mentioned above, seen in fetal telencephalic cells cultured in the presence of NGF [11].

A reduction in neuronal death occurring after a fimbria-fornix transection was reported by Hefti [40] and by Williams *et al.* [41] as a result of a continuous intraventricular infusion of NGF. Similar rescue effects by NGF on septal cholinergic neurons following lesions of the septo-hippocampal pathway was seen by Kromer [42] and have subsequently been confirmed in detailed analyses using immunohistochemical markers for these neurons [43, 44].

The effects of NGF on survival and fibre outgrowth of basal forebrain neurons in the fetal rat have been studied in culture by Hartikka and Hefti [45]. Survival effects by NGF on cholinergic neurons from the septum, the striatum as well as the nucleus basalis were found. In contrast, the length and degree of branching of nerve fibres increased in response to NGF only in the septal cultures [45].

In order to circumvent the possibility that NGF is increasing the proportion of cells with visible cholinergic traits rather than acting as a survival factor in culture of fetal brain cells, Arimatsu and collaborators [46] retrogradely labelled neurons in the septum projecting to the hippocampus by fluorescent latex microspheres. After labelling, the septum was dissociated and the number of labelled cells surviving in culture with and without NGF was determined. In the presence of NGF significantly more labelled neurons were seen, demonstrating a true survival effect by NGF on septal neurons. It thus appears that NGF can regulate the transmitter synthesizing enzyme, the fibre formation as well as the survival in responding CNS neurons, all in accordance with the effects of NGF in responsive peripheral neurons.

DEVELOPMENTAL REGULATION OF NGF AND NGF-R IN THE CNS

Several studies have described the developmental appearance of NGF in the brain and spinal cord. Whittemore *et al.* [21] reported that NGF mRNA is present only at trace concentrations in the fetal rat brain and increases postnatally, approximately

twenty-fold, until three weeks after birth, when adult levels had been reached. In contrast, the sensitive two-site enzyme immunoassay [21] indicated that the NGF protein is present in the rat brain already prenatally at levels higher than half of the adult levels. In fact a prenatal peak in NGF protein concentration in total brain at embryonic day 17 (E 17) was noted [21] and has since been confirmed in repeated ELISA tests (Lärkfors and Ebendal, unpublished observations). The reason for the apparent discrepancy during prenatal stages between detectable NGF protein levels and very low NGF mRNA levels is not known. The dramatic postnatal increase in NGF mRNA and NGF protein has been documented also in the hippocampus [47, 48] and the neocortex [47]. The increase in NGF protein paralleled that of the NGF mRNA and suggests that at this period of development the NGF level is regulated at the level of mRNA transcription or mRNA stability. Moreover the level of NGF in the basal forebrain closely followed the developmental increase in the hippocampus and cortex of NGF, as did the level of choline acetyltransferase of the basal forebrain [47, 48]. The close correlation between NGF levels and the levels of ChAT suggests a causal relationship. Another developmental curve was obtained for NGF in the cerebellum where levels of NGF mRNA and NGF protein gradually fell from postnatal day 10 until adult stages [47].

The surprisingly high levels of NGF protein in the fetal brain, preceding the increase in NGF mRNA referred to above [21] and also seen by Thoenen and collaborators [48] in the hippocampus at E 17 may suggest a distinct, early function of NGF in CNS development. The immunohistochemical finding of NGF in the fetal brain [29], demonstrating staining of many fibre systems in the E 15 rat fetus, may relate to such a function.

Also the developmental changes in the NGF receptor have been examined in the CNS. Binding of radiolabelled NGF to sections of the chick embryo suggested the early expression of NGF-R not only in the classical target neurons for the NGF protein but also in a variety of other cells, in particular skeletal muscle cells and cells of the lateral motor column of the spinal cord [49]. Strong binding was also seen in the dorsal horn indicating binding to sensory nerve fibres entering the spinal cord from the dorsal root ganglia. A transient nature of the NGF binding to the lateral motor column was documented [49] with strongest labelling seen at E 6 concomitant with binding of NGF to skeletal muscles. Also the ventral roots and spinal nerves leaving the spinal cord extensively labelled in the E 8–12 chick [49], indicating some early, unknown function of NGF in the building of the early CNS and its contacts with peripheral effector organs. A subsequent paper from the same authors extends the observation on NGF binding in the chick embryo, eye and cranial ganglia [50]. An early labelling of the mantle zone in the brain stem and spinal cord was seen. Also the rhombic lips and the tectum were labelled at E 6 as was the retinal ganglion cell layer [50].

This unexpectedly widespread, transient appearance of the NGF-R in the developing CNS has been confirmed also in the rat using immunohistochemistry to detect the NGF-R protein [51, 52]. As in the chicken, motoneurons of the lateral motor column were amply positive for the NGF-R during the first postnatal week, as were cells in the cerebellum and the visual system. Neurons in the basal forebrain (the septal nucleus and the nucleus basalis) were also stained from E 15 [51, 52], as expected from their NGF-R expression in the adult rat brain [32–34]. Also the olfactory tract and glomerular layer of the olfactory bulb was positive for the NGF-R [51]. The developmental expression of NGF-R immunoreactivity in the retina and cerebellum

(Purkinje cells, granule cells of the external granule layer) has been confirmed also in the primate CNS [53].

In the course of normal development, the drastically, as suggested from the binding and NGF-R immunohistochemical data, NGF-R expression in the CNS is not decreased, as examined by RNA blot hybridization [38]. In the basal forebrain even an increase in NGF-R mRNA has been shown [54]. The RNA blot analysis has, not unexpectedly, revealed a complex regulation of the NGF-R, and different areas in the brain show their own time-dependent pattern of expression [55].

The recent cloning of the chicken NGF-R [38, 56] has also allowed studies of the developmental regulation of the NGF-R mRNA expression in the chick embryo. In accordance with the binding studies using radiolabelled NGF [49], *in situ* hybridization experiments showed that the motoneurons of the spinal cord expressed the NGF-R early in development [38], as did myoblasts [38]. The functional significance of the early, transient expression of the NGF-R is not known: a detailed study of the lateral motor column in the chick embryo given daily NGF injections between E 3 to E 9 did not reveal any differences in the number of motoneurons compared to the control embryos [57].

Likewise, examination of NGF uptake and retrograde transport by motoneurons in newborn rats did not result in any increases in motoneuron size or increases in axon outgrowth or neurotransmitter enzyme (ChAT), nor in prevention of cell death following axotomy [58]. Possibly the apparent lack of effects is because the NGF receptors on the motoneurons are of the low-affinity type [37]. It is generally believed that the classical neurotrophic effects by NGF is mediated by the high-affinity NGF receptor, which may be the same protein as the low-affinity receptor but linked to a co-operating protein or the cytoplasmic side of the plasma membrane of the NGF-responsive neurons. However, several questions remain to be answered before the signal-transducing mechanism of the NGF–NGF-R interaction will be well understood.

The function of the early NGF-R expression in developing CNS may have implications for the possible future clinical use of NGF. Indeed, some striking growth effects by NGF have been reported on peptidergic neurons and on the Mauthner neurons in the CNS of tadpoles of the frog *Xenopus laevis* [59].

ALTERATIONS OF NGF AND NGF RECEPTOR LEVELS FOLLOWING CNS LESIONS

Denervation or transplantation will cause an increase in detectable NGF activity in some peripheral tissues [60]. This raises the question whether regions of CNS will also respond by altered NGF production, NGF stability or local accumulation of NGF.

Early studies indicated that noradrenergic sympathetic fibres will grow into the hippocampal formation following lesions of the fimbria-fornix [reviewed in 15 and 61]. This effect was suggested to be mediated by NGF [13, 62]. Evidence for this was obtained by Springer and Loy [61] when blocking some of the sympathetic fibre ingrowth by injecting antibodies to NGF prior to the fimbria-fornix transection. This finding is in contrast to the experience of limited success from many other attempts to block NGF in the brain by giving anti-NGF antiserum [3].

Direct determinations of the content of NGF in cholinergically denervated hippocampi has shown that there is indeed a local increase in the NGF protein by both bioassay [62] and by two-site enzyme immunoassay [63–67]. The increase in NGF level is about 50% [63–67], seems to be specific for the fimbria-fornix lesion as opposed to sham or entorhinal cortex lesions [63, 66, 67], does not involve a regulation by sympathetic fibres from the superior cervical ganglion [63–65, 67], and peaks one to two weeks after the fimbria-fornix intervention [64–67]. Interestingly, the increase in NGF protein in the adult rat brain is not preceded by any corresponding alteration in the level of NGF mRNA [21, 25, 65]. In contrast, the neonatal rat hippocampus responded with increases in the NGF level accompanied by similar increases in NGF mRNA [66]. The reason for the discrepancy between the response to fimbriectomy in the neonatal and adult hippocampus is not known, but suggests some developmental differences in the regulation of NGF mRNA in the hippocampus.

Also, suction lesions of the neocortex in adult rats have been found to result in increased levels of NGF, in this case in the basal forebrain and striatum [67]. The increase was maximal at two weeks after lesion in the nucleus basalis and after four weeks in the striatum. Several mechanisms could account for the lesion-induced increase in NGF, one being a local response to macrophage released interleukin-1 by analogy with NGF up-regulation in the sciatic nerve following lesion [68]. In fact, preliminary evidence suggested that interleukin-1 increased NGF mRNA in dissociated cultures of the perinatal rat hippocampus [69].

Also the NGF-R expression in CNS is regulated by lesions [70, 71]. In adult peripheral nerve, the NGF receptor has been found to reappear (after its developmental decrease, see references above) following a nerve crush [72, 73]. An increase in the NGF-R mRNA and the number of NGF-R positive cholinergic interneurons was found by Gage and collaborators [70] in the adult rat striatum following NGF infusion. The effect may be a lesion-induced re-expression of the NGF-R on the cholinergic interneurons or a ligand-induced up-regulation of the expression of the NGF-R. The molecular mechanism for the regulation of the NGF-R gene needs to be examined further.

Lesion-induced NGF receptor re-expression may also account for the NGF-R immunoreactivity seen in Purkinje cells of the adult rat cerebellum after colchicine treatment [74]. A striking reappearance of NGF-R mRNA expression in spinal cord motoneurons has recently been found in the adult rat following a crush lesion of the sciatic nerve [71]. An up to 12-fold increase in NGF-R mRNA was found one to two weeks after the lesion. The finding is intriguing and relates to the possible function of NGF in the motoneurons of the spinal cord, both during development and regeneration of axons in the adult. As discussed above no evidence is yet at hand to support a function for NGF in these neurons [57, 58] despite their expression of the NGF receptor. The problem is nevertheless one worthy of careful study, especially considering a similar case, the expression of NGF-R in the goldfish retina ganglion cells. In this system, NGF is retrogradely transported only after a prior lesion [75]. NGF in ng concentrations per ml has been found to stimulate neurite outgrowth in the goldfish retina only after a priming lesion or after 1–2 weeks of *in vitro* culture [76], during which time the NGF-R may become expressed.

AGE-RELATED CHANGES IN THE LEVELS OF NGF AND ITS RECEPTOR IN THE BRAIN

The possibility of age-related alterations in the levels of NGF has been examined by RNA blot analysis of the brain of aged Sprague-Dawley rats [21]. It was found that the NGF mRNA in total brain of 12–17 months old rats was reduced by 10–20% compared to the levels seen in the adult brain. Another analysis, of the NGF mRNA in forebrain of 28-month old Fischer 344 rats demonstrated a reduction by 50% [23] compared to 6-month old adults. The NGF protein in the hippocampus of the aged Fischer rats were also significantly decreased to levels 60% of that found in the adult (6-month old) Fischer rats by the two-site NGF enzyme immunoassay [23]. In contrast, no significant decreases in the level of NGF protein were found in the cortex during ageing.

In addition to showing a decrease in the NGF protein in the hippocampus during ageing, substantial differences were indicated between the NGF level of rats of different strains. Thus, adult Fischer rats showed up to three times higher levels of NGF in the hippocampus (3.2–3.6 ng/g tissue) [23, 77] than adult Sprague-Dawley rats. This suggests a genetic influence of the local NGF level in the brain.

Also alterations in the level of NGF-R protein or in the number of NGF-R bearing neurons in the aged rat brain has been described. Koh and Loy [78] described a substantial loss of NGF receptors in the basal forebrain of rats between 10 and 30 months of age based on NGF-R immunohistochemistry. The age-related decrease in NGF-R was indicated to be in part due to cell dysfunction rather than to cell death. Similarly, a reduced NGF binding capacity was found in the hippocampus and basal forebrain but not in the cerebellum of aged rats (26-month old) without any noticeable changes in K_d (6×10^{-9}M) [79], suggestive of a loss of a number of NGF receptors rather than changed affinity for the ligand. A loss of NGF-R immunoreactivity from neuron somas and in particular from the distal portion of the dendrites of basal forebrain neurons in aged rats have been described [80], again indicating a critical role for NGF reception in the declined function of the ageing brain. In contrast to the lowered binding and immunoreactivity of the NGF receptor, its mRNA has not been found to have changed in the aged rat forebrain [55].

NGF IN RELATION TO ALZHEIMER'S DISEASE

One major function of NGF in the brain is clearly to support maintenance and performance of the cholinergic neurons of the basal forebrain. Such a function is now well established based on the distribution of NGF-producing cells in the cerebral cortex and hippocampus, the presence of NGF receptors in the basal forebrain cholinergic neurons, the uptake and retrograde transport of NGF in these neurons and their responses to NGF. The pronounced loss of these neurons and the lowering of ChAT activity in Alzheimer's disease has focused attention to NGF either as a causal factor for the disease or, perhaps more likely, as a way of clinically stimulating the affected cholinergic neurons via their natural neurotrophic factor. A review on this topic has appeared [81]. A cholinergic deficit has been a principal finding in Alzheimer's disease and may be responsible for the memory deficits characteristic of this progressive disease. It was concluded by Hefti and Weiner [81] that an increase in

the availability of NGF to the cholinergic neurons in the basal forebrain of the afflicted Alzheimer patient may retard the degeneration and improve the clinically manifest dysfunction of these NGF-sensitive neurons in the septal area and the nucleus basalis of Meynert (Fig. 3).

In order to pursue a strategy for the use of NGF to treat Alzheimer's disease it would be of great value to learn more about the regulation of NGF and its receptor in the normally ageing human brain and in the brain of Alzheimer patients. Also the levels of NGF in brains of patients with Down's syndrome, sharing some neurodegenerative traits with Alzheimer's disease, would be interesting. Little information regarding these topics are available at present. In part this may be due to the lack of a suitable two-site enzyme immunoassay for human NGF. Additional problems are the difficulties in obtaining biologically active, recombinant human NGF. No doubt further research will change the scene.

RNA-blot analysis of the human cerebral cortex in 5 cases of senile dementia of the Alzheimer type did not show significant losses in levels of NGF mRNA [25]. Very recently the levels of the NGF-R mRNA was also examined in Alzheimer brains. No evidence was found that the NGF-R mRNA had decreased in the basal forebrain of five patients who died from diagnosed Alzheimer's disease [82]. The authors conclude that NGF might be therapeutically useful since it can be anticipated that the cholinergic neurons in the basal forebrain will respond to applied NGF since the receptors are still expressed. Such a clinical experiment remains to be performed and the outcome evaluated.

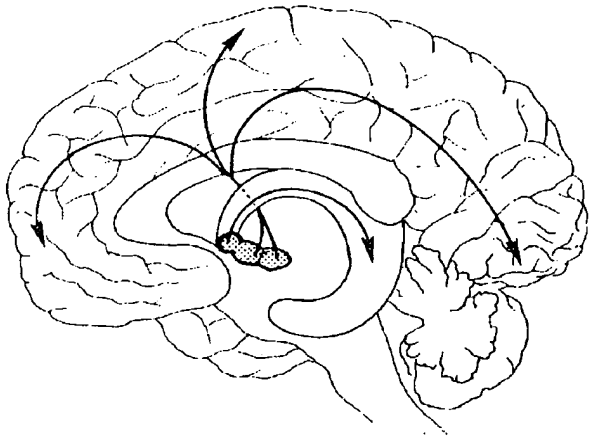

FIGURE 3. The NGF-responsive cholinergic projections from the human basal forebrain (medial septum nucleus, nucleus of the diagonal band of Broca and nucleus basalis of Meynert) to the cerebral cortex and hippocampus. The depicted system is implicated to function in memory processes and is severely affected in Alzheimer's disease. Figure redrawn from Ref. 92.

Animal experiments have indicated that such an approach might be feasible. Behaviourally impaired, aged rats served as a model for the memory-impaired Alzheimer patient [83] and it was shown that a continuous intraventricular infusion of NGF over a period of four weeks in part reversed the atrophy of basal forebrain cholinergic neurons and, most important, to some extent improved the retention of a spatial memory task. A previous study [84] has indicated also that NGF infusion will

facilitate recovery of behaviour after a fimbria-fornix lesion, affecting the basal forebrain cholinergic neurons.

POSSIBLE CLINICAL APPLICATIONS OF NGF TO NEURODEGENERATIVE DISEASE

NGF may be of clinical use in Alzheimer's disease since the trophic action of NGF is directed towards the severely affected cholinergic neurons of the forebrain.

Another neurodegenerative disease where NGF might find a clinical application is Parkinson's disease. It is important to point out that NGF does not act on the dopaminergic neurons of the substantia nigra [8, 30]. However, as described below, NGF is known to affect the adrenal medulla [85]. Such tissue has been used in clinical trials of a transplantation therapy with adrenal medullary tissue grafted to the striatum of Parkinson patients [86].

In rat experiments [85], the infusion of NGF in the area of the chromaffin graft in the dopamine-denervated striatum was studied. NGF greatly enhanced the number of surviving cells in the graft and their transformation to a neuronal phenotype. Moreover, NGF stimulated local adrenergic nerve growth and improved the performance of the rats in a test of apomorphine-induced rotational behaviour [85]. Thus, the NGF treatment effectively counteracted some of the symptoms resulting from the induced parkinsonism in rats. An approach with NGF-treatment of adrenal medullary grafts also in human Parkinson patients seems feasible but clinical tests remain to be performed.

A major concern in clinical applications of NGF to treat neurodegeneration in the brain is the way to administer the trophic factor. The blood–brain barrier will most likely comprise a hindrance for NGF given intravenously to enter the CNS. To mimic the normal actions of NGF a local administration either in the target areas for the cholinergic projections or near the NGF-responsive neuron somas seems more logical. This can be achieved by infusions of NGF to the patient's brain via canula devices.

Other possibilities include the use of low molecular weight peptides with NGF-like activity penetrating the blood–brain barrier or substances acting pharmacologically to enhance the endogenous NGF production in the brain of the patient. All these possibilities remain to be tested in animal experiments. It thus seems urgent to learn more about the regulation of NGF synthesis in the brain, and the mechanisms by which the effects of NGF is transduced into the responding neurons, in order to design suitable substances that could mimic the actions of NGF.

A further interesting possibility is to transplant NGF-producing cells to the brain in order to establish a local supply of NGF. Such cells may be genetically modified [87] prior to transplantation into the CNS. Several fibroblast cell lines producing increased amounts of NGF have recently been established after infection with a retroviral vector [88] or transfection [89] with plasmid constructs containing the gene encoding for NGF. Such cells have then been used for transplantation into the rat brain to examine the effects evoked by the NGF-producing cells on cholinergic neurons in the brain of the hosts. In one of the studies the cells were grafted after a fimbria-fornix lesion [88]. It was shown that the cell graft produced enough NGF in the host brain to prevent the degeneration of cholinergic neurons that would otherwise have taken place and that the genetically modified cells were significantly more effective to prevent degeneration

than the uninfected parental cell line. In addition to the increase in number of ChAT-positive cells, an increase in acetylcholinesterase (AChE)-positive fibres and cell staining was observed together with a sprouting of AChE-positive fibres near the NGF-producing graft [88].

Another study [90], based on the NGF-transfected mouse 3T3 cell line designated 3E [89], also describes striking cholinergic growth responses after grafting of the transfected cells to the brain of immunosuppressed rat hosts. The 3E cells used for grafting are stably expressing a 25-fold increased NGF-release compared to the parent cell line and condition their growth medium so that the classical NGF-induced fibre halo around explanted embryonic ganglia [2, 60] is easily demonstrated as is the drastic increase of NGF mRNA in the 3E cells [90]. The 3E cells were grafted under several different conditions, either as a cell suspension or trapped in a collagen matrix. When grafted in the collagen matrix to the intact striatum of adult rats, the NGF-producing cells but not a cell-free matrix or a collagen matrix with the parent 3T3 cells, evoked a markedly increased density of cholinergic nerve terminals around the implant as evaluated by AChE immunohistochemistry [90], a good cholinergic marker in this part of the brain [3, 45, 90]. In addition, AChE-positive fibres invaded only the 3E cell containing collagen implant.

Also a more complex co-grafting experiment was reported [90]. In this case, the NGF-producing 3E cells were grafted together with NGF-dependent fetal cholinergic neurons from the basal forebrain [91] into the cerebral cortex of adult rats that had previously received ibotenic acid injections to reduce the cholinergic projections to the cortical areas. Four weeks after the co-grafting, the survival of the fetal cholinergic neurons was significantly higher with the 3E cells than with the 3T3 parental cells [90]. Cholinergic nerve fibre growth was also greatly enhanced. In addition to the effects on the grafted fetal neurons, the NGF-producing cells were found to stimulate intrinsic, local AChE fibres in the cortex [90], again lending support to the responsiveness of cholinergic neurons to NGF.

Thus, considerable evidence from animal experiments support the feasibility of transplanting genetically modified, NGF-producing cells to the brain in order to achieve growth effects in cholinergic neurons. Several questions need to be answered before this approach can be clinically useful. In particular the possibility of cell grafts being tumourigenic needs to be examined. Also the immunological problems of introducing foreign cells that have been manipulated in culture for extended periods of time should be examined. Finally, the type of human cell most suited for such grafting purposes needs to be carefully studied.

Irrespective of which of the possibilities outlined above will eventually be found most effective to meet neurodegenerative disease, it seems highly likely that the time is ripe for a serious and careful clinical evaluation of the possible beneficial effects of NGF on the afflicted human brain.

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